Introduction

As memories are acquired and consolidated, a cascade of intracellular events involving a variety of signaling molecules occurs, ultimately resulting in gene induction and protein synthesis necessary for long-term memory formation.

(Davis & Squire, 1984)

Moreover, many of the protein products encoded by IEGs act as messengers in coupling short-term neural activity with long-term functional and structural changes by acting as transcription factors and regulating gene expression.

It has been suggested that changes in IEG expression are involved in information storage and long-term memory consolidation.

(Goelet, Castelucci, Schaecher, & Kandel, 1986)

Differential induction of c-Jun and Fos-like proteins in rat hippocampus and dorsal striatum after training in two water maze tasks

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Immediate early genes (IEGs)
- c-fos
- c-jun
→ markers of cell activation

(Morgan and Curran, 1991 and Sagar et al., 1988)
In experiments using post-training intracerebral drug infusions
- we have previously shown that
  - glutamate
  - NMDA receptor activation
  - lipid platelet-activating factor (PAF)
    - are required for memory consolidation in
      - hippocampal-dependent water maze tasks
      - dorsal striatal-dependent water maze tasks

(Packard & Teather, 1999, Packard & Teather, 1997, Teather, Packard, & Bazan, 2001)

Glutamate, NMDA receptor activation, and PAF-mediated signal transduction cascades
→ induce the rapid expression of several IEGs, including c-fos and c-jun, in both
  hippocampal and striatal cell preparations

(Bading et al., 1993, Liste et al., 1995, Squinto et al., 1989 & Vaccarino et al., 1992)

Hypothesis
Expression of IEG products may be selectively induced in a region specific manner following training in hippocampal-dependent and dorsal striatal-dependent memory tasks

To test this prediction we examined
- the number of cells immunopositive for Fos-like proteins and c-Jun in the dorsal hippocampus and dorsal striatum of rats following training in
  - a hippocampus-dependent spatial water maze task (→ hidden escape platform)
  - a dorsal striatal-dependent cued water maze task (→ visible escape platform)

Materials and methods

**Animals**
- 24 male Long–Evans rats (300–380 g) (Charles River Breeding Laboratories; Wilmington, MA)
- 2–3 per cage
- standard environmental conditions (room temperature: 20–22 °C; relative humidity: 55–60%;
  light/dark schedule: 12/12 h)
- free access to water and lab chow
- All procedures were carried out during the initial 6 h of the light period of the light/dark cycle
- Experiments were carried out in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals

**Antibodies**
- anti-c-Jun polyclonal antibody (sc-45; Santa Cruz, Santa Cruz, CA)
- anti-c-Fos polyclonal antibody (sc-1695; Santa Cruz)
  dilution of 1:5000 → minimal non-specific staining
Spatial and cued water maze tasks

- **Water (25±2°C)**

- **Hidden Platform**: 8 cm in diameter
- **20 cm**: Depth
- **Ø8 cm**: Diameter

Pre-set criterion
- Rats had to achieve two consecutive trials with retention latencies under 8 s; and could take no more than 10 trials to reach this criterion (10 min)
- Due to the time-dependent nature of this study (i.e., Fos/Jun levels were examined 90 min after task acquisition) these criteria were used to keep training times within a limited range (10 ± 3 min)

Three rats (2 visible platform task; 1 hidden platform task) were removed from the study due to excessive thigmotaxic behavior: swimming around the perimeter of the pool.

In addition, one rat did not acquire the visible platform task to criterion and was discarded from the study.

Spatial training trial
- The latency to mount the escape platform was recorded and used as a measure of task acquisition

Cued training trial
- The latency to mount the escape platform was recorded and used as a measure of task acquisition

Control animals
- **Naive control rats**
  - For estimates of basal IEG protein expression
- **Yoked-control (i.e. free-swimming) rats**
  - For an assessment of possible changes in IEG-IR that result from swimming behavior, sensorimotor stimulation, stress, & other factors not related to the tasks
**Immunocytochemistry**

- sodium pentobarbital (70 mg/kg, i.p.)
- heparinized saline
- 4% paraformaldehyde solution prepared with 0.1 M phosphate buffered saline (PBS; pH 7.4)

- Perfusion

- Coronal brain matrix

- Incubate in 1% sodium borohydride in PBS for 30 min
- Rinse in 0.1 M PBS three times for 2 min each
- Two 5 min rinses in 0.1 M Tris-buffered saline (pH 7.6) containing 0.1% Triton X-100 (TBST)
- Incubate 1% normal goat serum in TBST for 1 h

- Free floating section

- Overnight incubation in primary antibody in TBST containing 1%
- Several washes in TBST
- Incubate with biotinylated secondary antibody in a TBS solution containing 1% BSA

- Washing

- Avidin–biotin–peroxidase immunohistochemistry solution diluted 1:100 with 1% BSA in TBS

- Two rinses for 15 min each in TBS
- 0.02% 3,3-diaminobenzidine tetrahydrochloride (DAB) solution containing 1/1000 volume of 3% H$_2$O$_2$ (6 min)
Data evaluation

- The nomenclature and nuclear boundaries utilized in this study were based on the atlas of Paxinos and Watson (1986).

Cell nuclei expressing levels of DAB reaction product above tissue background were automatically counted by a computerized image analysis system (Image Pro Plus 4.0; media Cybernetics, CA).

Cell count data were analyzed by ANOVA followed by Schaeffe’s post hoc analysis were appropriate.

Data were considered significantly different when $p < .05$.

Results

Acquisition of spatial and cued water maze tasks

A. Spatial water maze tasks
   - Asymptotic performance by trial 7
   - Mean (±SD) of trials to criterion: $6.67 ± 1.03$

B. Cued water maze tasks
   - Asymptotic performance by trial 8
   - Mean (±SD) of trials to criterion: $8.17 ± 1.16$

Learning-induced immunoreactivity in dorsal hippocampal subfields: Fos-like

Fos-like immunoreactivity in coronal sections through the stratum pyramidale cells of the CA1 region of the dorsal hippocampus

Scale bar = 50 μM

Fold-increase in Fos-like immunopositive cells in stratum pyramidale cells of the CA1 region of the dorsal hippocampus

Data are presented as the ratio of Fos-like immunoreactive cells
Learning-induced immunoreactivity in dorsal hippocampal subfields: c-Jun

- c-Jun immunoreactivity in stratum pyramidale cells of the CA1 region and CA3 pyramidal cells of the dorsal hippocampus

- One-way ANOVA analyses revealed significant main effects of group for the number of Fos-like labeled cells in the lateral \( F(3, 20) = 29.76; p < .0001 \), and in the medial \( F(3, 20) = 33.76; p < .0001 \) regions of the dorsal striatum
- Scheffe’s post hoc analyses indicated that all groups that experienced the water maze (i.e., cued, spatial, and yoked-controls) had increased Fos-like-IR in both of these striatal regions \( (p's < .05) \)

Discussion

- c-Jun and Fos-like protein expression patterns differ in a structure- and task-dependent manner during memory consolidation in the rat
- training in a spatial task → ↑ c-Jun protein expression in the CA1 & CA3 subfields of the dorsal hippocampus
- → ↑ Fos-like protein expression in the CA1

These findings suggest that Fos-like protein expression occurs in both the medial and the lateral regions of the dorsal striatum due to non-mnemonic factors (e.g., swimming behavior, sensorimotor function, and stress)
training in a cued
→ patches of cells expressing c-Jun
reactivity in the posteroverentral aspect of the dorsal striatum

The observed hippocampal induction of Jun/Fos proteins during the early phase of memory consolidation demonstrated in the present study is generally consistent with previous findings
→ For example
  → ↑ Fos & Jun proteins levels in the chick brain 1 h after training in a passive avoidance task (Freeman & Rose, 1995)
  → ↑ c-Fos protein levels, in the dorsal CA1 region of rats 60 min post-training in an operant task (Bertaina-Anglade, Tramu, & Destrade, 2000)
  → c-Fos expression in the CA3 following training in a spatial radial maze task (He, Yamada, & Nabeshima, 2002)

Although our results revealed Fos-like-IR to be increased in the CA, especially in rats that acquired the spatial task, we found no learning-specific increase in Fos-like protein expression in the CA3.
→ This may be due to differences in the tasks used in these studies, as we used aversive water maze training whereas the previously mentioned studies used appetitive tasks

Swim-yoked control rats
→ ↑ IEG protein product expression in the dorsal hippocampus (at lower levels than rats trained in the spatial task)
Our findings suggest that swimming behavior per se, sensorimotor function, or stress may have induced this activation

Alternatively, IEG activation in the swim-yoked controls may result from hippocampal processing of spatial information, even in the absence of reinforced escape learning
→ It might suggest
  → The formation of a spatial map, or perhaps latent learning (e.g., learning about the environment) in the yoked-controls increases hippocampal IEG activity

Discussion
Immediate early gene expression in dorsal hippocampus
→ rats trained in a spatial task
→ ↑ Fos-like-IR in the CA1 of the dorsal hippocampus
→ ↑ c-Jun-IR in the CA1 and CA3 regions

Swim-yoked control rats
→ ↑ IEG protein product expression in the dorsal hippocampus (at lower levels than rats trained in the spatial task)
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Alternatively, IEG activation in the swim-yoked controls may result from hippocampal processing of spatial information, even in the absence of reinforced escape learning
→ It might suggest
  → The formation of a spatial map, or perhaps latent learning (e.g., learning about the environment) in the yoked-controls increases hippocampal IEG activity
Discussion

**Immediate early gene expression in dorsal striatum**
- In all rats that acquired the spatial & cued water maze tasks, and in swim-yoked controls
  - ↑ number of Fos-like-immunopositive cells in the medial and lateral aspects of the dorsal striatum
- Such non-specific task enhancement in c-Fos expression has been previously shown in rats that acquired an operant task (Bertaina-Anglade et al., 2000)

As no differences in Fos-like activity were found in these regions, the increased cellular activation likely resulted from the swimming experience and/or latent learning of information unrelated to task
- Consistent with this suggestion, running on a treadmill induces widespread c-Fos activation in the entire dorsal striatum (Liste, Guerra, Caruncho, & Labandiera-Garcia, 1998)

In contrast to the general increase in Fos-like-IR
- small patches of c-Jun-IR were selectively observed in the posteroventral aspect of the dorsal striatum of rats that underwent training in the cued task
- Four of six rats trained in the cued task had such patches, whereas none of the rats trained in the spatial task displayed these patches

Anatomical studies indicate
dorsal striatum is a heterogenous structure, both in terms of
- its neurochemically distinct intrinsic compartments (i.e., matrix and striosomes) (Graybiel, 1990)
- its regionally ordered topographic connectivity with other cortical/subcortical structures (Alexander, DeLong, & Strick, 1986)

Lesions of the medial dorsal striatum
→ transient impairment in acquisition of spatial behavior in the water maze (Whishaw, Mittleman, Bucnch, & Dunnett, 1987)
- it has been suggested that the medial dorsal striatum mediates a “cognitive” form of memory similar to that associated with the hippocampus (Devan & White, 1999)

Other findings indicate
- in a spatial water maze task acquisition is normal in rats with medial dorsal striatal damage (Packard & McGaugh, 1992)
- lesions of the medial dorsal striatum do not impair hippocampus-dependent spatial memory in a radial maze (Sakamoto & Okaichi, 2001)
we found no evidence for a learning-specific effect in the medial dorsal striatum, although clearly further research is necessary to examine this hypothesis for a range of IEG's.

The present finding of selective induction of c-Jun IR within a posteroventral region of the dorsolateral striatum following cued learning is consistent with previous studies that have used intracerebral drug infusions to examine the role of this particular striatal location in memory.

Specifically, post-training infusion of
- dopamine agonists
- platelet-activating factor antagonists
- glutamate
- NMDA receptor antagonists AP5
have each been found to selectively modulate habit memory in the cued water maze task when administered into the posteroventral region of the dorsolateral striatum.

Although we did not carry out double-labeling studies in the current study, making it impossible for us to precisely determine which cell type(s) was responsible for the increased c-Jun expression, the cells appeared to be medium-sized spiny neurons (from counterstaining studies using cresyl violet).

Similar patches of have been shown to occur (c-Fos rather than c-Jun) as a result of amphetamine administration; these patches were termed striosomal clusters (Hebb & Robertson, 1997).

General conclusions
The present findings indicate that c-Jun and Fos-like proteins expression is up-regulated in a task-dependent and brain structure-specific manner shortly after acquisition of hippocampus-dependent and dorsal striatal-dependent learning.

The findings are consistent with the double dissociation of the mnemonic functions of the hippocampus and dorsal striatum that have been previously demonstrated using brain lesion and pharmacological techniques (Packard & Knowlton, 2002).
The present increase in Fos-like and c-Jun proteins after training is consonant with a prevailing molecular hypothesis of memory consolidation. Stimuli that trigger the encoding of long-term memory initiate molecular cascades that modulate the expression of immediate early genes (IEGs) which in turn modulate the expression of late response genes, culminating in enduring structural and functional alterations in areas involved in memory formation (Goel et al., 1986).

Interestingly, glutamate, NMDA receptor activation, and PAF-mediated signal transduction cascades induce the rapid expression of several IEGs, including c-Fos and c-Jun (Bading et al., 1993, List et al., 1995, Squinto et al., 1989, Vaccarino et al., 1994) and mediate hippocampal- and striatal-dependent memory during the early consolidation period (Packard and Teather, 1997, Packard and Teather, 1999, Teather et al., 1999, 2001).

Alterations in synaptic activity via glutamatergic and lipid-mediated neurotransmission can induce IEG expression that could ultimately pay a role in information storage by regulating the function of later response genes encoding for various structural or synaptic proteins.

The task-dependent increases in IEG immunoreactivity observed in the present study in the hippocampus (c-Jun and Fos-like) and dorsal striatum (c-Jun) may ultimately contribute to mechanisms of synaptic plasticity that encode information within these two separate memory systems.